SUB B1

G4 [24] 25. (Amended) The transgenic animal of claim [23] 24, wherein said animal carries a mutation in a *daf-18* gene.

Add the following new claim 26.

G5 26. The method of claim 5, further comprising the step of testing said identified compound in a diabetic or obesity mouse model system.

REMARKS

The invention currently claimed relates to methods for the identification of a compound capable of modulating the expression or activity of a *daf-18* or PTEN gene. Such compounds are useful for ameliorating or delaying impaired glucose tolerance conditions or obesity, or increasing the longevity of a cell or organism.

Support for the Amendments

To expedite allowance, Applicants have canceled original claims 5 and 6 and have amended original claim 4 (present claim 5) to specify that the claimed DAF-18 transgenic animal screening methods are carried out in nematodes. In addition, Applicants have canceled present claim 24 (original claim 23) and amended present claim 23 to specify a transgenic nematode whose cells contain a mammalian PTEN transgene. For the record, Applicants disagree with the basis for the rejection of these claims, and Applicants

reserve the right to pursue all canceled subject matter in this or future continuing applications.

Support for the amendment of present claim 5 is found in the specification at pages 196-199. Support for the amendment of present claim 23 is found in the specification at pages 119-120. Newly added claim 26 specifies a method for identifying a compound that modulates the expression or activity of a *daf-18* gene, further comprising the step of testing a compound identified in nematodes in a diabetic or obesity mouse model system. Support for this claim can be found in the specification at pages 201-202.

In addition, the claims have been renumbered, as required by the Examiner, and the specification has been amended to correct typographical errors and to correct the sequence identification numbers. These errors are regretted. No new matter is added by any of the amendments.

Summary of the Office Action

Claims 1-25 stand rejected, under 35 U.S.C. § 112, first paragraph. This rejection is addressed below.

Sequence Listing

As required by the Examiner, Applicants provide herewith a Sequence Listing which is believed to satisfy the requirements of 37 C.F.R. 1.821 through 1.825.

Claim Objections

The numbering of the claims as originally filed is objected to because more than one claim is numbered as claim 2. In response to this objection, Applicants have amended the numbering of the claims to provide each claim with a unique number.

Rejections under 35 U.S.C. § 112, First Paragraph

Claims 1-25 stand rejected, under 35 U.S.C. § 112, first paragraph, based on the assertion that Applicants' specification does not enable the presently claimed *daf-18* or PTEN screening methods, nor does it enable transgenic animals containing a transgene encoding a mammalian PTEN polypeptide.

This rejection of claims 1-25 is based on the assertion in the Office Action that Applicants have not demonstrated the efficacy of the claimed screening methods and that these methods would not be predicted to work because (i) the specification fails to provide guidance for an assay for measuring *daf-18* or PTEN gene expression or activity; (ii) the specification fails to provide guidance for making a transgenic nematode or mouse expressing a PTEN gene; (iii) the specification fails to describe the regulatory regions of *daf-18* and PTEN gene promoters; (iv) the specification provides no correlative teachings as to the interaction and effect of the human PTEN gene in a nematode cellular/genetic environment; and (v) the specification fails to show that *daf-18* or PTEN genes are associated with the onset of impaired glucose tolerance conditions, obesity, or longevity.

These bases for the rejection are respectfully traversed.

The first basis for the § 112 rejection relates to the statement in the Office Action that the specification fails to provide guidance for an assay for measuring *daf-18* or PTEN gene expression or activity. This basis for the rejection is respectfully traversed.

It is Applicants' position that a variety of techniques for assaying DAF-18 and PTEN expression and activity are indeed provided in the present specification. As evidence of this assertion, Applicants direct the Examiner's attention to pages 197-198 of the specification, where methods to measure the level of expression and activity of DAF-18 and PTEN are provided. Such methods include standard Northern or Western blot analyses of DAF-18 or PTEN expression, as well as the measurement of the phosphorylation state of targets of these proteins.

With respect to carrying out Northern or Western blot analyses, applicants point out that those skilled in the art of molecular biology can readily design probes to detect *daf-18* or PTEN RNA levels and can also generate antibodies to such proteins for use in Western blots. For example, probes to measure *daf-18* RNA expression levels can be designed and utilized in a Northern blot analysis according to the methods of Brown, as described in Current Protocols in Molecular Biology (Ausubel et al., John Wiley and Sons, Inc., 1994, pages 4.9.1 to 4.9.14), provided as Exhibit A. In addition, antibodies to DAF-18 or PTEN can be generated as described at pages 53-137 of Harlow and Lane (Antibodies: A Laboratory Manual, Cold Spring Harbor, NY, Cold Spring Harbor Press,

1988), provided as Exhibit B. These antibodies can be used to measure PTEN or DAF-18 protein levels by Western blot analyses, as also described by Harlow and Lane (supra) at pages 471-510, provided as Exhibit C. Each of the above techniques is considered a standard method of molecular biology, and each may be carried out utilizing the *daf-18* and PTEN sequences described in Applicants' specification and Li et al. (Science 275:1943-1947, 1997), respectively.

Alternatively, phosphorylation assays for measuring PTEN expression or activity may be used to identify modulatory compounds in the presently claimed screens. Such phosphorylation assays were similarly established before the filing of the present application. Prior to Applicants' filing date, for example, it was known in the art that PTEN levels could be monitored based on PTEN's ability to dephosphorylate the second messenger, phosphatidylinositol 3,4,5-triphosphate, as described in the attached publication by Maehama et al. (J. Biol. Chem. 273:13375-13378, May 29, 1998). One skilled in the art of molecular biology could simply use the Maehama technique to measure the phosphorylation level of phosphatidylinositol 3,4,5-triphosphate, as a means to determine whether a candidate compound modulated the expression or activity of PTEN.

Furthermore, Applicants submit that, once the above assays are carried out, the assay results are readily interpretable to skilled practitioners. For example, a candidate compound which increases *daf-18* or PTEN gene expression in cells or test animals is

detected by an increase in RNA or protein levels when such a sample is compared to a control which did not receive the candidate compound. Similarly, a candidate compound that increases PTEN activity in cells or test animals may be identified using a similar assay format and detecting the ability of the compound to increase the phosphorylation level of phosphatidylinositol 3,4,5-triphosphate over that observed in parallel controls. In short, a number of standard assays may be utilized to carry out the claimed screening methods, and these assay approaches are provided in Applicants' specification. This basis for the rejection may also be withdrawn.

The second basis for the § 112 rejection focuses on the concern that the specification fails to provide guidance to make and use any transgenic nematode or mouse expressing a PTEN gene. This rejection is respectfully traversed.

Applicants first point out that present claim 23 of the invention has been amended to specify a transgenic nematode whose cells contain a transgene encoding a mammalian PTEN gene. Applicants submit that the generation of a nematode containing a transgene encoding mammalian PTEN involves only straightforward techniques. In particular, the PTEN gene is publicly available and may be readily obtained, for example, from the GenBank sequence database (see Exhibit D). With this gene in hand, transgenic nematodes may be readily generated using the techniques provided in Applicants' specification at page 38, where the successful production of a nematode expressing a *daf-3* transgene is described. Based on the amendment of claim 23 and the teaching in

Applicants' specification describing transgenic nematode production, Applicants respectfully request that the second basis of the rejection be withdrawn.

The third basis for the § 112 rejection involves the assertion in the Office Action that the specification fails to describe the regulatory regions of nematode *daf-18* and mammalian PTEN gene promoters. This rejection is respectfully traversed.

As an initial matter, Applicants submit that it is relatively straightforward for one skilled in the art to obtain the regulatory sequences of a gene. For example, standard PCR and genomic library screening techniques or computer searches of genomic sequence databases may be used to obtain regulatory elements of a desired gene.

In addition, Applicants point out that it is not necessary, nor do the claims of the present invention require, that expression of a PTEN or DAF-18 polypeptide be directed by its endogenous promoter. Quite to the contrary, as attested to in the attached Declaration of Dr. Gary Ruvkun, any number of regulatory elements may be used to express these polypeptides, as recited in claims 1-25.

As discussed by Dr. Ruvkun, a variety of *C. elegans* promoters may be used to express a heterologous gene, such as DAF-18 or PTEN, in a cell, and this has been demonstrated by the inventors and other researchers. In particular, as outlined in the Ruvkun Declaration, the *C. elegans ins-1* promoter has been shown to be capable of successfully driving expression of the human insulin gene.

Alternatively, any number of other promoters may be used for DAF-18 or PTEN

expression. For example, tissue-specific promoters may be utilized for this purpose. This strategy has been successfully achieved in *C. elegans* previously, as also attested to in the Declaration of Dr. Gary Ruvkun. Dr. Ruvkun states that an *age-1* cDNA has been expressed in *C. elegans* using tissue-specific promoters. For example, *age-1* has been expressed specifically in neurons (using the *unc-14* promoter), in muscle (using the *unc-54* promoter), and in intestines (using a gut specific *ges-1* synthetic promoter), and has been expressed ubiquitously (using the *dpy-30* promoter) in a dauer-arrested *C. elegans age-1* mutant. Accordingly, any of the above promoters may be used to direct expression of DAF-18 or PTEN in a cell.

A further example of the ability of a mammalian gene to be expressed from a heterologous promoter in *C. elegans* is also provided in the Declaration of Dr. Ruvkun. There, Dr. Ruvkun describes the successful expression of the mammalian FKHRL1 gene under the direction of the *C. elegans daf-16* promoter. This result, like the above results, demonstrates that mammalian genes may be expressed from heterologous promoters, and may even be expressed from promoters which are derived from entirely different species. Moreover, because FKHRL1 and the human insulin gene are members of the insulin signaling pathway in mammals, Dr. Ruvkun's results further demonstrate that expression of genes involved in regulating glucose metabolism do not have to be expressed from their endogenous promoters to function properly in an animal.

Finally, Applicants point out that the upstream regulatory region of mammalian

PTEN is publicly available and may be readily obtained, for example, from the Entrez sequence database, as evidenced by Exhibit E. In view of the above comments, Applicants respectfully request that the third basis of the rejection be withdrawn.

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The fourth basis for the § 112 rejection pertains to the statement in the Office Action that the specification provides no correlative teachings as to the interaction and effect of human PTEN in a nematode cellular/genetic environment. This basis for the rejection is respectfully traversed.

On this issue, Applicants refer the Examiner to pages 104-117 of the specification, where evidence is presented regarding the structure and function of DAF-18 and PTEN. As discussed in the specification at pages 108-110, the *C. elegans daf-18* gene exhibits sequence homology to the human PTEN gene, and this homology is particularly striking within the phosphatase domain. Consistent with this structural similarity, these genes appear to share a functional role. PTEN possesses lipid phosphatase activity and acts to decrease PI3K lipid products in response to insulin signaling in human cells. *Daf-18*, in similar fashion, is part of the insulin signaling pathway in *C. elegans,* and *daf-18* mutants bypass the requirement for *age-1* PI3K signaling, again supporting the similarity between these genes and their ability to function in an interchangeable manner. In addition, on this point, Applicants refer the Examiner to the Ruvkun Declaration. There, Dr. Ruvkun notes that another mammalian insulin signaling gene, FKHRL1, a gene that shares structural and functional similarities with a *C. elegans* member of the insulin signaling

pathway, the *daf-16* gene, has been demonstrated to functionally complement *daf-16* mutants. Based on the structural and functional similarities between *daf-18* and PTEN and Applicants' demonstration that other such similar mammalian insulin-signaling genes function in a *C. elegans* genetic and cellular environment, Applicants submit that it is entirely reasonable to predict that PTEN would function in a nematode and be useful for screens to identify compounds that modulate either *daf-18* or PTEN activity. Applicants respectfully request that this fourth basis for the rejection be withdrawn.

The final basis for the § 112 rejection pertains to the assertion in the Office Action that the specification fails to show that any *daf* or PTEN gene is associated with the onset of impaired glucose tolerance, obesity, or longevity. This rejection is respectfully traversed.

Taken in turn, Applicants first submit that the evidence relating to the role of *daf* genes and the PTEN gene in insulin signaling is striking. First, with respect to PTEN, Applicants refer to the discussion above, describing experiments which demonstrate that PTEN is involved in the regulation of insulin signaling in human cells (Maehama et al., supra). This evidence directly indicates that, as a regulator of insulin signaling, PTEN is involved in impaired glucose tolerance conditions.

In addition, Applicants point out that the *daf* genes of the present invention, including *daf-18*, are involved in pathways for regulating glucose metabolism, the members of which are conserved from *C. elegans* to mammals. As described at pages 2-5

of the specification, two signaling pathways are required for the regulation of metabolism and dauer arrest in *C. elegans*: the DAF-2 insulin signaling pathway, and the DAF-7 TGF-β signaling pathway. *C. elegans* which are deficient in either the DAF-7 or the DAF-2 signaling pathway arrest at the dauer stage, indicating that both pathways are essential for regulating metabolism and dauer arrest.

As attested to in the Declaration of Dr. Gary Ruvkun, the TGF-β and insulin signaling pathways in humans and *C. elegans* share a large number of family members. For example, *C. elegans* gene products involved in the regulation of metabolism through the TGF-β signaling pathway include DAF-7, DAF-4, DAF-1, DAF- 8, DAF-14, and DAF-3. Mammalian family members of this same pathway have also been identified. For example, DAF-7 is a particular subtype of the TGF-β superfamily; DAF-1 and DAF-4 are *C. elegans* members of the type I and II TGF-β receptor families, respectively; and DAF-3, DAF-8, and DAF-14 are *C. elegans* members of the Smad family of proteins.

The TGF-β signaling pathway acts in concert with the insulin signaling pathway to regulate metabolism and dauer arrest. Mammalian members of the insulin signaling pathway include insulin, the insulin receptor, PI-3 kinase, PDK1 kinase, AKT/PKB kinase, and the particular forkhead proteins FKHR, FKHRL1, and AFX. Again, *C. elegans* orthologs of many of these mammalian gene products have been identified. For example, DAF-2 is the *C. elegans* ortholog of the human insulin receptor superfamily; AGE-1 is the *C. elegans* ortholog of human PI-3 kinase; PDK-1 is the *C. elegans* ortholog

of human PDK1; DAF-18 is the *C. elegans* ortholog of human PTEN; *C. elegans* AKT-1 and AKT-2 are orthologs of human AKT kinase; and DAF-16 is the *C. elegans* ortholog of human FKHR, FKHRL1, and AFX.

Moreover, Applicants point out that human proteins of the insulin pathway can function in *C. elegans*, interacting with *C. elegans* proteins to regulate metabolism. For example, as attested to in the Declaration of Dr. Gary Ruvkun, the human FKHRL1 gene, a member of the mammalian forkhead protein family that is a DAF-16 ortholog, can be expressed under the control of the *C. elegans daf-16* gene promoter in *daf-16* mutant *C. elegans*. When so expressed, the human FKHRL1 gene functionally complements the *daf-16* mutant.

Each of the above findings emphasizes the fact that human and *C. elegans* insulin signaling pathways are functionally similar, and that the *daf* genes which are members of this pathway are indeed involved in the regulation of impaired glucose tolerance conditions.

Similarly, with respect to insulin conditions involving obesity, Applicants again submit that a number of lines of evidence indicate that the *daf* and PTEN genes play roles in obesity conditions and that the presently claimed screening methods could indeed be exploited for the identification of anti-obesity compounds.

On this issue, Applicants first direct the Examiner's attention to the specification at page 62, lines 10-14. There, the specification describes a 14 year old diabetic insulin-

resistant patient, who was morbidly obese. This patient carried the same insulin receptor mutation as a *C. elegans daf*-2 mutant (*e1391*) described in the present specification, demonstrating the association between *daf* genes and conditions involving obesity in humans.

In addition, Applicants direct the Examiner's attention to the Declaration of Dr. Gary Ruvkun. There, Dr. Ruvkun points out that dauer arrest of various insulin signaling pathway mutants can be rescued by expression of a protein which complements the mutation, and that such a rescue also results in the loss of fat in the rescued animals. For example, *C. elegans daf-2* mutants are normally dauer-arrested and exhibit increased fat accumulation when compared to their wild-type counterparts. When the *daf-2* mutants are rescued from dauer arrest (for example, by mutation of the *daf-16* or *daf-18* gene) and therefore from an impaired glucose tolerance condition, they also exhibit lower fat accumulation levels. Similar results are observed in *C. elegans age-1* mutants. These mutants also exhibit increased fat accumulation as dauers, and, upon rescue from dauer arrest by expression of the wild-type AGE-1 protein, similarly exhibit lower fat accumulation levels.

Paragraph 8 of Dr. Ruvkun's Declaration presents yet another set of experiments demonstrating that *daf* genes are associated with the onset of impaired glucose tolerance conditions involving obesity. There, Dr. Ruvkun reveals the effects of the elimination of serotonin in *C. elegans* on dauer arrest and fat accumulation. As stated in the

Declaration, serotonin is not made in *C. elegans* mutants lacking the *cod-5/tph-1* gene, a gene which normally encodes the serotonin synthesis protein, tryptophan hydroxylase. Such *cod-5/tph-1* mutant animals exhibit phenotypes which are characteristic of *daf* mutants: up to half of the *cod-5/tph-1* mutant animals arrest at the dauer stage and accumulate large amounts of fat. In addition, there is also a lower level of expression of DAF-7 in *cod-5/tph-1* mutants. These similarities, which indicate that there is a serotonin input to the *daf-7* pathway. Serotonin also acts upstream of the insulin signaling pathway. For example, a *daf-16* mutation suppresses the dauer arrest and increase in fat accumulation of *cod-5/tph-1* mutants, again providing evidence that the presently claimed screens are useful for identifying compounds involved in obesity. It is well known that the serotonergic pathway in humans is a target of therapeutics used to treat obesity, and the involvement of serotonin in the *daf* pathway indicates that *daf*-related screens would similarly be useful for isolating anti-obesity therapeutics in Applicants' claimed system.

With respect specifically to the role of PTEN, Applicants refer the Examiner to Figs. 38A-38F of Applicants' specification, where it is demonstrated that the *C. elegans* homolog of PTEN, *daf-18*, modulates the level of fat accumulation in *C. elegans*. The data presented in this figure shows that *daf-18* suppresses the fat accumulation phenotype of an *age-1* null mutant, providing direct evidence that *daf-18* is involved in the regulation of fat levels in nematodes. Applicants submit that, due to the structural and functional similarity between DAF-18 and PTEN (discussed above) and the known role

of PTEN in the regulation of insulin signaling in human cells, PTEN, like *daf-18*, would be predicted to regulate fat accumulation and could successfully be used in the presently claimed screens for obesity-related compounds.

Moreover, Applicants submit that the relationship between impaired glucose tolerance conditions and fat accumulation in mammals is well established in the medical literature. The inability of a mammal to regulate metabolism results in a shift of its metabolism away from burning energy and toward metabolism of fat. The metabolism of fat, in turn, leads to other conditions in mammals, including obesity.

For all of the above reasons, Applicants submit that the relationship between the *daf* genes, as well as the PTEN gene, and impaired glucose tolerance conditions leading to obesity has been established, and this basis for the rejection may be withdrawn.

Finally, to address the contention in the Office action that the specification fails to show that *daf* genes regulate longevity in nematodes, Applicants refer the Examiner to pages 103-107 of the specification, where evidence of the role of *daf* genes in longevity is described. There, Applicants indicate that weak *daf-2* and *age-1* mutants that do not arrest at the dauer stage nevertheless live much longer than their wild-type counterparts. In addition, a *daf-18* mutation supresses the long life span of the *daf-2* and *age-1* mutants. These results demonstrate that the insulin signaling pathway, of which *daf-2*, *age-1*, and *daf-18* are members, can modulate longevity. The specification further states that *age-1* null mutants are characterized by their longevity phenotype. These *age-1* null mutations

are suppressed by *daf-18(e1375)*. Again, these results clearly demonstrate that *daf* genes, including *daf-18,* play a role in the regulation of longevity in nematodes.

With respect to the role of PTEN in longevity, Applicants again refer the Examiner to the specification at pages 103-107, where the ability of *daf-18* to regulate longevity is detailed, as described above. Applicants submit that, due to the functional similarity between DAF-18 and PTEN and the ability of mammalian genes (such as FKHRL1) to function in the DAF pathway in *C. elegans*, one skilled in the art would predict that PTEN also functions to regulate longevity and could be used in the presently claimed screens.

In sum, Applicants have demonstrated that the genes of the DAF pathways, as well as the PTEN gene, function to regulate impaired glucose tolerance conditions, fat accumulation, and longevity, providing strong evidence that screens involving these genes and pathways would successfully identify compounds involved in such conditions. Applicants submit that this final basis for the § 112 rejection may be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

Enclosed is a petition to extend the period for replying for two months, to and including October 27, 1999.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 26 October 1999

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